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Influence of α-Thalassemia Trait on Spleen Function in Sickle Cell Anemia Patients With High HbF

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Spleen function was studied in a group of 20 Kuwaiti SS patients (aged 2–12 years), using ^{99m}Tc-labeled tin colloid scintigraphy. They were screened for the α-thalassemia determinants which are prevalent in the Arabian Peninsula [−α (3.7 kb) deletion, α2-globin gene polyadenylation signal (AATAAA → AATAAG) mutation, and 5′ IVS-I splice junction pentanucleotide (GAGGTGAGG → GAGG) deletion] with a combination of polymerase chain reaction and allele-specific oligonucleotide (ASO) hybridization techniques. The patients were divided into three groups depending on the result of their colloid uptake. Group I consisted of 7 patients (35.0%) with normally visualized spleens, Group II consisted of 5 (25.0%) with partial visualization, and in Group III there were 8 (40.0%) in whom the spleen was not visualized at all. The significant distinguishing features among those in Groups I and III were mean corpuscular volumes (MCVs) of 74.1 ± 5.1 and 90.1 ± 6.6 fl ($P < 0.0001$) and mean corpuscular hemoglobins (MCHs) of 22.4 ± 2.7 and 27.5 ± 4.0 pg ($P < 0.05$), respectively. The overall frequency of α-thalassemia determinants in the study was 35.0%; however, the frequencies in Groups I, II, and III were 57.1, 30.0, and 18.8%, respectively. α-Thalassemia trait, therefore, appears to be associated with normal splenic function in these patients. © 1996 Wiley-Liss, Inc.

Key words: splenic scintigraphy, mean corpuscular volume (MCV)

INTRODUCTION

Sickle cell anemia (SCA) is usually a mild disease among Kuwaiti Arabs, as in patients from Eastern Saudi Arabia (1). This is not surprising since the original settlers of Kuwait migrated from the Najd province of East Central Saudi Arabia in the 17th/18th centuries (2,3). The β^S mutation in this part of the Arabian Peninsula is usually found on chromosomes of haplotype 31 [Saudi Arabia/India (SAI)] background, and homozygotes have hemoglobin F (HbF) levels of about 15% to > 30%. The latter is believed to be a major ameliorating factor in the clinical course of SCA (4,5). However, there are a few patients who, in spite of an elevated HbF, run an atypically severe course with frequent painful crises and fulminant bacterial infections. The factors that characterize this subset have not been fully elucidated.

The spleen, because of its peculiar microvasculature and sluggish circulation, bears the brunt of the pathology [rigidity of the red blood cell (RBC) membrane and consequent recurrent vaso-occlusion and infarction] in SCA. Therefore, quite early (within the first 2 years) in the life of the patient, functional asplenia occurs, followed later (usually within the first decade) by fibrotic autosplenectomy (6,7). The natural history of this process has not been well documented in patients with high HbF levels. It has, however, been reported that they maintain their splenic function until an older age, compared with patients with low fetal hemoglobin (8,9).

In the present study, splenic function was evaluated using ^{99m}Tc-labeled tin colloid and heat-denatured RBC scintigraphy. The patterns obtained were correlated with the following parameters: age, frequency of painful crises, frequency of bacterial infection necessitating hospitalization, hematologic data: hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin

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(MCH), and concentration (MCHC), HbF levels, β^S haplotype, and α-globin genotypes. Plasma ferritin was also determined to find out if iron deficiency played a role in some of the patients with microcytosis and hypochromia.

MATERIALS AND METHODS

The subjects of this study were SS patients being followed in the Pediatric Hematology Clinics of Mubarak Al-Kabeer and Al-Amiri Hospitals in Kuwait. Informed consent was obtained from the parents of all patients. Clinical histories and patients' charts were reviewed to document the frequency of acute events necessitating hospitalization, especially painful crisis and bacterial infections, since the diagnosis of SCA was made. All the patients except one, who had acute splenic sequestration, were in steady state at the time of the study. None had any other chronic illness apart from SCA.

About 5 ml of blood was obtained by venipuncture into vacutainers with EDTA anticoagulant. Complete blood counts were obtained with an electronic cell counter (Coulter S). Fresh hemolysate was prepared from each sample and subjected to isoelectric focusing (IEF) (10) and cation exchange high-performance liquid chromatography (11,12) to quantitate Hbs A, S, F, and A_2. Patients with patterns not consistent with HbSS were excluded from the study.

The fresh blood was centrifuged and the plasma separated and kept frozen at −70°C until plasma ferritin was determined using a radioimmunoassay method (Biorad). DNA was extracted from leukocytes by the method of Poncz et al. (13). β^S-globin gene cluster haplotypes were determined by hybridization of amplified DNA, dot-blotted on nylon membranes, with enhanced chemiluminescence (ECL)-labeled synthetic oligonucleotide probes. These probes are specific for certain mutations in the $^G\gamma$- and $^A\gamma$-globin gene promoters of chromosomes with the Benin (19), SAI (31), and Bantu (20) haplotypes. Details of the methodology have been previously described (13–15).

The α-globin genotypes were determined by screening for the −α (3.7 kb) deletion using a modified Baysal and Huisman polymerase chain reaction method (16). All the samples were also screened for the α2 polyadenylation signal mutation (AATAAA → AATAAG) and the 5′ IVS-I splice junction GAGGTGAGG → GAGG pentanucleotide (5 nt) deletion, which are prevalent in this region, using hybridization of amplified DNA with ECL-labeled specific synthetic oligonucleotides as previously reported (15).

Liver/spleen scintigraphs were performed with ^{99m}Tc-labeled tin colloid (Amersham) in all patients (17,18). Radionuclide images of the posterior, left lateral, and anterior views of the splenic area were obtained in all studies. The results were graded as normal if the splenic visualization was of the same intensity as the liver image (Group I), partial if there was a decrease in the splenic image (Group II), or no visualization (Group III). Patients in Groups II and III were re-studied at least 2 days later, but within 2 weeks, with heat-denatured ^{99m}Tc RBC (Amersham) scintigraphy (18,19).

Data are presented as means ± SD except where otherwise stated. Student's t-test, analysis of variance (ANOVA), or Chi-square test were used, as appropriate, to test statistical significance of differences between mean values or proportions in different groups. Analysis was with Statgraphics version 6.0 IBM-compatible PC software.

RESULTS

There were 20 SS patients (13 boys, 7 girls) aged 2–12 years (mean, 6.4 ± 2.5 years) in the study. Their individual hematological and other data are shown in Table I. Seven (35.0%) patients had normally visualized spleens (Group I), five (25.0%) had partial visualizaton (Group II), and eight (40.0%) were not visualized (Group III) on ^{99m}Tc tin colloid liver/spleen scans. All those in Group II and one in Group III had normal splenic visualization on ^{99m}Tc heat-denatured RBC scan (Fig. 1).

There was a sequential increase in MCV (74.1 ± 5.1, 84.3 ± 14.0, and 90.1 ± 6.6 fl, respectively) and MCH (22.4 ± 2.7, 25.8 ± 3.9, and 27.5 ± 4.0 pg, respectively), but a slight decrease in total Hb and HbF from Groups I to III. ANOVA showed that the differences between the values in the three groups were only significant for MCV ($P < 0.01$). However, when the mean values were compared between Groups I and III there were significant differences for MCV ($P < 0.0001$) and MCH ($P < 0.05$).

β^S haplotyping showed that all patients were homozygous for SAI haplotype except patient 10 (Table I), who was compound heterozygous for Benin and SAI haplotypes, and patient 19, who was homozygous for the Bantu haplotype. The latter had a severe clinical course, with multiple acute episodes, mostly pain crisis and severe anemia. She also had the lowest HbF (0.4%) in the whole group. At the time of the study, she was on chronic transfusion therapy. Her hematologic values were not included in computing the means for the study population and are not shown in Table I.

α-Globin genotype was successfully determined in all 20 patients. Of these, nine (45.0%) had a normal gene complement (αα/αα), seven (35.0%) were α-thal-2 heterozygotes ($-\alpha^{3.7}/\alpha\alpha$), two (10.0%) were α-thal-2 homozygotes ($-\alpha^{3.7}/-\alpha^{3.7}$), one (5.0%) was a compound heterozygote for α-thal-2 and the IVS-I pentanucleotide deletion ($-\alpha^{3.7}/-\alpha^{5nt}\alpha$), and one (5.0%) was a simple heterozygote for the pentanucleotide deletion ($\alpha\alpha/\alpha^{5nt}\alpha$). Thus the frequency of α-thal determinants in the study population was 35.0%. However, in Group I, the fre-

TABLE I. Individual Haematological and Other Data*

	No.	Sex-age (years)	β-Globin haplotype	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb F (%)	α-Globin pattern	Pain crises	Infection
Group I	1	F-5	31/31	8.8	80.4	19.8	29.2	24.0	$-\alpha/\alpha\alpha$	+++	–
(n = 7)	2	M-7	31/31	9.9	73.2	23.8	26.5	24.8	$-\alpha/\alpha^{-5nt}\alpha$	+	+
	3	M-12	31/31	10.2	74.9	20.2	27.0	29.4	$-\alpha/\alpha\alpha$	++	–
	4	M-3	31/31	8.1	79.9	25.1	31.5	28.0	$\alpha\alpha/\alpha\alpha$	–	–
	5	F-6	31/31	8.2	65.9	18.8	28.5	15.2	$-\alpha/-\alpha$	+	–
	6	M-3	31/31	10.3	74.6	25.7	31.5	27.6	$-\alpha/\alpha\alpha$	–	–
	7	M-9	31/31	8.9	70.1	23.1	32.9	15.0	$\alpha\alpha/\alpha^{-5nt}\alpha$	+	–
Group II	8	M-6	31/31	9.4	92.1	27.6	31.6	28.0	$\alpha\alpha/\alpha\alpha$	+	+
(n = 5)	9	M-7	31/31	8.2	94.0	29.7	27.5	28.0	$\alpha\alpha/\alpha\alpha$	+	–
	10	M-3	31/19	9.1	77.0	26.2	32.2	19.9	$\alpha\alpha/\alpha\alpha$	+	–
	11	M-3	31/31	8.9	95.3	26.2	27.0	26.2	$-\alpha/-\alpha$	+	–
	12	F-7	31/31	10.6	63.0	19.2	25.4	14.7	$-\alpha/\alpha\alpha$	++	–
Group III	13	M-8	31/31	8.1	93.7	28.4	30.6	24.8	$-\alpha/\alpha\alpha$	+	+
(n = 8)	14	F-4	31/31	10.6	97.1	24.0	24.8	20.4	$-\alpha/\alpha\alpha$	+	–
	15	F-8	31/31	8.7	97.1	31.3	32.2	13.9	$\alpha\alpha/\alpha\alpha$	+	++
	16	M-10	31/31	7.3	81.5	21.0	25.8	16.1	$-\alpha/\alpha\alpha$	++	–
	17	M-9	31/31	9.3	87.2	27.4	31.2	17.2	$\alpha\alpha/\alpha\alpha$	–	–
	18	F-9	31/31	9.4	89.0	28.0	31.4	24.2	$\alpha\alpha/\alpha\alpha$	–	+
	19[a]	F-4	20/20	nd[b]	nd	nd	nd	0.4	$\alpha\alpha/\alpha\alpha$	+++	+
	20	M-6	31/31	9.6	94.5	32.5	34.4	30.6	$\alpha\alpha/\alpha\alpha$	++	–

*Hospital admissions: +++ = >10; ++ = 5–10; + = 1–4.
[a]On chronic transfusion therapy (see text for details).
[b]nd, no data.

quency was 57.1%, in Group II, 30.0%, and in Group III, 18.8%. The difference in the distribution between Groups I and III is significant ($\chi^2 = 5.5$, $P < 0.05$).

None of our patients had plasma ferritin values suggestive of iron deficiency. The values ranged from 21 to ~1,000 ng/ml, the highest being in the patient with Bantu haplotype on chronic transfusion therapy.

Six (30.0%) patients (five boys, one girl, aged 3–12 years) had spleens palpable 3–13 cm below the left costal margin. The 3-year-old boy with the largest spleen (13 cm) had acute splenic sequestration at the time of the study; otherwise the others were in steady state. There was no significant difference between the mean age, Hb, MCV, MCH, or HbF of this group compared with the values in those without palpable spleens. They were all homozygous for the SAI haplotype except the boy with acute splenic sequestration, who was a compound heterozygote for SAI and Benin haplotypes. α-Globin gene status showed that three were α-thal-2 heterozygotes ($-\alpha/\alpha\alpha$), two had a full complement ($\alpha\alpha/\alpha\alpha$), and one was an α-thal-2 homozygote ($-\alpha/-\alpha$). In two patients there was normal colloid uptake, while in three the spleen was partially visualized on colloid scan, but showed normal denatured RBC uptake. One patient showed no uptake on both colloid and denatured RBC scintigraphy.

One patient each in Groups I and II had been hospitalized because of one episode of pneumonia, while in Group III, four (50.0%) had had severe infections (pneumonia, osteomyelitis, lung abscess, and pyelonephritis). Frequencies of infections and pain crises are shown in Table I. One patient (patient 16, 8 years old) in this group has had pneumonia (three episodes), lung abscess, and pyelonephritis. Most patients in the three groups had been hospitalized on several occasions because of severe pain crisis.

DISCUSSION

Splenic dysfunction is believed to contribute significantly to the predisposition to bacterial infections in SCA. However, its role in patients with high HbF, who generally have a milder clinical course, has not been adequately investigated. It is interesting therefore that in the present study, the patients who have had recurrent severe bacterial infections are mostly in the group with poor splenic function, as shown by labeled colloid and heat-denatured RBC scans.

The conventional scintigraphic method of demonstrating functional asplenia has been the labeled-colloid uptake technique. However, in a previous study from Kuwait, Owunwanne et al. (17) showed that in a group of seven SCA patients, aged 6–20 years, in whom the spleen was either not demonstrable or partially so on colloid uptake, it was well visualized on heat-denatured RBC scan. This observation has also been reported in a patient (with idiopathic thrombocytopenia, post-splenectomy) who had residual spleen tissue that was not demonstrable on colloid uptake, but was well defined on heat-denatured

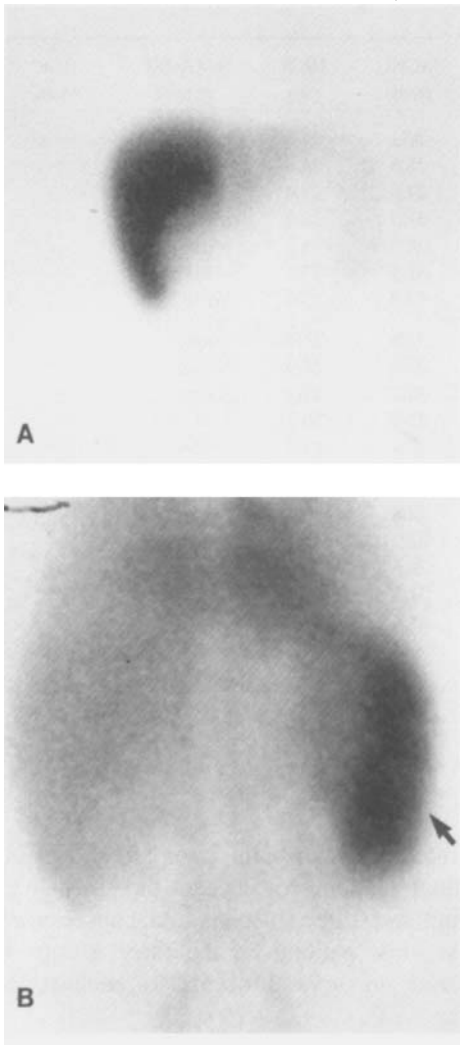

Fig. 1. A: ^{99m}Tc-tin colloid liver/spleen scintigraph, which shows a well-visualized liver, but no spleen. B: Heat-denatured ^{99m}Tc-RBC scintigraph on the same patient. Distinct splenic uptake (black arrow) can now be seen.

RBC uptake. This probably reflects the different mechanisms of uptake of colloid and denatured RBCs. Splenic uptake of radiopharmaceuticals is accomplished by the phagocytic function of reticuloendothelial cells in removing particulate matter from the circulation while the uptake of denatured RBCs is accomplished more by filtration within the splenic red pulp (17,19). The present cross-sectional study shows that there is a progression from 1) normal uptake of colloid to 2) partial or no uptake of colloid, but normal denatured RBC uptake, and finally to 3) the severe situation in which both scanning techniques are negative. It is not known whether this is the normal sequence of events in individual SCA patients; a prospective study, currently under way, will hopefully confirm this.

The splenic dysfunction in SCA develops from a blockage of the small inter-endothelial slits in its sinuses by the rigid or sickled red cells (7). It is plausible that this process depends, to some extent, on the size of the cells. It is therefore interesting that the most significant differentiating factor among the patients with non-visualized spleens on colloid and denatured RBC uptakes in the present study is the MCV. While none of the patients had evidence of iron deficiency, the frequency of α-thal determinants among patients with normal visualization was 57.1% and only 18.8% in those with no visualization.

Babiker et al (8) studied two groups of SCA children from Saudi Arabia; one group of 25 from the Southwestern region had low HbF (5.6–10.0%) while the other group of 10 from the Eastern region had high HbF (16–25%). Eighty-four percent of the first group had no splenic colloid uptake, while in the second group 80% had normal uptake. In another study from Eastern Saudi Arabia, Mallouh et al. (9) found that among 15 SCA children, 13 had either normal or partially visualized spleens on colloid uptake. In our study, only 60.0% of the children had normal or partially visualized spleens on colloid uptake. It would be interesting to compare the frequencies of α-thal trait in the Kuwaiti and Saudi SCA populations.

Co-existent α-thalassemia is a recognized ameliorating factor in SCA patients with low HbF (19,20). It decreases the rate of hemolysis by decreasing the MCHC, thereby resulting in higher Hb, Hct, and RBC values (21). Complications such as leg ulcers, renal pathology, and strokes are fewer, but the frequency of other complications, e.g., osteonecrosis and retinopathy, may be increased (22–25). Overall survival may also be enhanced (23,26). The present study is probably the first to recognize an association of α-thal trait with preserved splenic function in SS patients with elevated HbF levels.

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